Apoptosis, p53, and Tumor Cell Sensitivity to Anticancer Agents

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Abstract

A widely held tenet of present day oncology is that tumor cells treated with anticancer agents die from apoptosis, and that cells resistant to apoptosis are resistant to cancer treatment. We suggest, in this review, that this tenet may need to be reexamined for human tumors of nonhematological origin, for two principal reasons: (a) cell killing has often been assessed in short term assays that are more influenced by the rate, than the overall level, of cell killing. This has tended to underestimate cell killing for cells not susceptible to apoptosis or having mutant p53; and (b) conclusions from experiments with normal cells transformed with dominant oncogenes have often been extrapolated to tumor cells. This does not take into account the fact that tumor cells have invariably undergone selection to an apoptotically resistant phenotype. In this review, we examine the impact of these two factors with particular emphasis on the influence of mutations in p53 on the sensitivity of tumor cells to DNA-damaging agents. We find that because wild-type p53 predisposes cells to a more rapid rate of cell death after DNA damage, particularly with normal or minimally transformed cells, that short-term assays have led to the conclusion that mutations in p53 confer resistance to genotoxic agents. On the other hand, if clonogenic survival is used to assess killing in cells derived from actual solid human tumors, then apoptosis and the genes controlling it, such as p53 and bcl-2, appear to play little or no role in the sensitivity of these cells to killing by anticancer drugs and radiation.

Introduction

During the past 10 years, interest of basic scientists and clinicians in the influence of programmed cell death, or apoptosis, on the sensitivity of tumors to anticancer treatment has risen and continues to rise dramatically. A major reason for this interest is that apoptosis is a defined program of cell death that is markedly influenced both positively and negatively by a variety of genes, many of which are mutated and/or dysfunctionally regulated in human cancers (1). Among the most important of these are the tumor suppressor gene p53 and members of the bcl-2 gene family (1, 2). The fact that apoptosis is a genetically defined pathway has led to two principal expectations: (a) that the genotype of the tumor will be predictive of the outcome of current anticancer therapy; and (b) that new therapies based on apoptosis will be superior to present-day anticancer treatments. The requirement for wild-type p53 for apoptosis after genotoxic damage caused by anticancer agents including irradiation has been well demonstrated, particularly in oncogenically transformed rodent cells and in tissues of lymphoid origin (3, 4). However, the influence of p53 and other genes on apoptosis in malignant tissues of nonhematological origin is by no means clear. There have also been reports indicating that apoptosis does not correlate with the total cell kill measured by other means following anticancer therapies. In this review, we will focus on tumor cells of nonhematological origin. In particular, we review critically the data underlying the hypothesis that these cancer cells when treated with radiation or chemotherapeutic drugs die of apoptosis, and that cells resistant to apoptosis are resistant to cell kill by anticancer therapy.

Many genes have been identified that affect the extent to which certain cell types undergo apoptosis during normal development and after pathological stress. Together with the assumption that apoptosis plays a major role in cell killing by DNA-damaging agents, these genetic studies have led to the present hypothesis that tumors with mutations in p53, high levels of bcl-2, or high ratios of bcl-2:bax should be resistant to cancer treatment (1, 2, 5). Because there is now a wealth of data from clinical studies in which outcome has been correlated with the status of these and other genes affecting apoptosis, this hypothesis would seem an easy one to test. However, a major problem with such analyses is that it is often impossible to separate treatment sensitivity from patient prognosis. For example, tumors with mutated p53 can be more anaplastic, can have a higher proportion of proliferating cells, can be more metastatic, and in general can have a more aggressive phenotype that similar tumors with wild-type p53 (6). This can lead to a worse prognosis for patients whose tumors have mutated p53 independent of treatment sensitivity (7). Having said this, there are numerous examples in the literature where p53 mutations (or high levels of p53 protein by immunohistochemistry) either do not affect patient prognosis (8, 9) or lead to better outcome after treatment (10, 11). In a comprehensive review of the clinical significance of p53 mutations in human tumors, Bosari and Viale (12) concluded (in 1995) that a definite answer could not yet be given to the question or whether p53 aberrations led to a more aggressive phenotype or to treatment resistance.

Apoptosis and Sensitivity to Anticancer Therapy: The Present View. As we point out above, because mutations in p53 or other genes may affect tumor aggressiveness and patient prognosis, it is difficult to obtain from clinical data an answer to the question of the role of p53 or of apoptosis in treatment sensitivity. However, experimental systems can be not only free of such biases, they can also use modern gene knockout, transgene, and other molecular techniques to answer the narrower question of: “Does the level of apoptosis and/or genes controlling apoptosis affect the sensitivity of cancer cells to killing by genotoxic agents?”

The present view is that this is the case (1, 2, 5, 13, 14). It has become widely accepted that cell death after DNA damage by anticancer agents is primarily the result of apoptosis, and that cells that can evade apoptosis will be resistant to cell killing. Often cited for this view, and in particular the role of mutated p53 in radiation and anticancer drug resistance, are pioneering studies with dominant oncogene-transformed normal fibroblasts from embryos of p53 wild-type (p53+/+) and p53 knockout mice (p53−/−) (15, 16), as well as highly significant associations of mutated p53 with drug resistance in the National Cancer Institute panel of 60 cell lines used for screening novel potential anticancer drugs (17).

However, despite the seemingly strong case that cells die from cancer treatment due to apoptosis largely controlled by wild-type p53,
several investigators have reported results that contradict this hypothesis, particularly when they have measured both apoptosis and overall cell killing by a colony-forming assay. For example, it has been reported that large changes in apoptosis do not lead to any changes in eventual cell killing (18–23), or that the status of p53 does not affect sensitivity to DNA-damaging agents. However, the present confusion can be largely resolved if two facts are borne in mind.

(a) Many investigators have used methods for assessing the extent of cell killing by anticancer drugs and radiation based on early functional changes (such as dye uptake), or on growth inhibition, rather than on colony formation. These assays can lead to incorrect assessments of overall cell kill, largely because they ignore kinetic differences in the manifestation of cell death.

(b) Conclusions derived from normal cells transformed with dominant oncogenes such as EIA and myc have been extrapolated to tumor cells. However, apoptosis, particularly the early apoptosis characteristic of cells of lymphoid origin and oncogene-transformed normal cells, is often an insignificant mode of cell death for the cells of the majority of solid tumors, irrespective of the status of genes such as p53 and bcl-2.

Both of these issues are explored in more depth in the following sections.

**Short-Term Assays Can Underestimate Overall Cell Killing.**

How should cell killing be measured after a toxic insult? This would not seem to be a particularly difficult problem; a dead cell has many distinct morphological features, loses metabolic functions, and fails to exclude dyes such as propidium iodide and trypan blue. If death is due to apoptosis, then a number of well-characterized features occur including chromatin condensation and fragmentation, formation of nucleosomal “DNA ladders,” and exposure of phosphatidyl serine in the outer cell membrane that can be detected with annexin V. Thus, dead cells can be readily identified, and their proportion in a population readily quantitated. However, identification of dead cells once they have died is not the problem. The problem is that cells do not die immediately after treatment; they can take hours to many days before dying, and this is highly dependent upon the cell type and the toxic agent being investigated (19, 24–26). Despite this, many investigators assessing cellular sensitivity to genotoxic agents measure viability by total population staining [the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or XTT3 assays] or assess the extent of cell death from the proportion of cells incorporating a dye excluded by live cells, such as trypan blue or propidium iodide, at short times (1–4 days) after treatment. As we point out below, this can lead to an underestimate of the overall level of cell killing.

The problem of correctly assessing the fraction of cells surviving a given treatment was solved for mammalian cells in the mid-1950s by Puck and Marcus (27), who developed the technique of cloning individual cells *in vitro*. The ability of a single cell to grow into a colony (usually defined as >50 cells) is an assay that tests every cell in the population for its ability to undergo unlimited division. It is, for mammalian cells, the exact counterpart of assays measuring bacterial or yeast survival after treatment with cytotoxic agents. The assay has become widely used for assessing the response to cytotoxic agents of cells *in vitro*, as well as cells in normal tissues and tumors *in vivo*. When the logarithm of the percentage of surviving cells determined by the clonogenic assay is plotted against the dose of agent used, a straight line (sometimes with an initial “shoulder” region) is usually obtained, implying an exponential relationship between dose and cell kill. Both for radiation and anticancer drugs, this “log cell kill” hypothesis for cell survival has been demonstrated under many different conditions to be effective in predicting the radiation or chemotherapy dose needed to cure experimental mouse tumors or multicellular spheroids (28–30).

Fig. 1 shows data illustrating the problem of using short-term assays to assess the influence of p53 on the sensitivity of cells to anticancer therapy. In this experiment, MEFs from wild-type (p53+/+) or p53 knockout mice (p53−/−) and transformed with EIA and ras (15) were exposed to different concentrations of etoposide for 1 h and assayed either by the XTT assay 1 day after exposure or by clonogenic survival after 8 days of incubation. Pooled data from two independent experiments are shown; bars, SE.

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Short-term assays can also be a problem *in vivo* as illustrated in Fig. 2. Here we have grown the EIA- and ras-transformed MEFs from wild-type (p53+/+) and p53 knockout mice (p53−/−) as tumors in severe combined immunodeficient mice and measured their response to a single dose of irradiation. Because the p53−/− cells undergo early apoptosis, leading to rapid and extensive tumor shrinkage, an assessment of tumor response by measuring tumor size within 2 weeks of irradiation would conclude that the p53−/− tumors were more radiosensitive. This is because the rate and/or extent of tumor shrinkage is largely governed by the rate of cell death rather than by the overall level of cell kill. However, growth delay, usually to 2–3 times treatment volume, is a way of assessing tumor response that is not affected by the rate of cell death (31, 32) and, as can be seen in Fig. 2, suggests that the initial apoptotic response observed in the p53 wild-type tumors has little or no effect on the final growth delay of these tumors.

The ability of cells to undergo unlimited proliferation as tested by their ability to form a colony has, therefore, become the “gold standard” for assessment of cellular sensitivity to cytotoxic treatments. It is, however, important to be precise about the meaning of this; it is the best way to assess the proportion of cells surviving a particular treatment under the experimental conditions used. It does not mean that all responses of tumors can be reliably predicted using a clonogenic assay of the cells treated *in vitro*. This was recently illustrated in studies of Waldman and colleagues and by us (22, 23). HCT116

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3 The abbreviations used are: XTT, 2,3-bis[2-methyl-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt; MEF, mouse embryo fibroblast.
human colon carcinoma cells isogenic for the p53 target gene $p21^{\text{wt}}$ (designated $p21^{+/+}$ or $p21^{-/-}$) had the same response when tested by clonogenic survival in vitro (although they differed greatly in their level of apoptosis under the same conditions). However, when tumors were grown in vivo from the same cells, those from $p21^{-/-}$ cells were much more sensitive to radiation as measured by growth delay than their wild-type counterpart. This was originally suggested as a failure of the clonogenic assay to correctly reflect tumor cell sensitivity (22, 33, 34). However, if clonogenic survival is assessed from the irradiated tumors, then there is close agreement between this assay and the in vivo growth delay assay for the two cell lines (23). The difference, therefore, between the in vitro and in vivo data are the result of different environmental conditions of the cells at the time of treatment.

When used with care, the clonogenic assay is probably the most reliable method for assessing cell killing after genotoxic agents (35). With this in mind, we can now ask the question of whether apoptosis is a reliable determinant of cell killing to radiation and anticancer drugs when assayed by clonogenic survival in vitro. Although there are many examples in the literature, particularly for cells of hematological origin, where apoptosis and cell killing assayed by clonogenic survival are well correlated, there are also many examples, particularly for cells of nonhematological origin, where this is not the case. Figure 3, for example, shows some of our own results in which the response of HCT 116 cells that are either $p21^{+/+}$ or $p21^{-/-}$ have been assessed both for apoptosis and for clonogenic survival under identical treatment conditions to radiation, to etoposide, and to bioreductive drug tirapazamine. It is clear from these data, and from other examples in the literature (18–20, 36), that the extent of apoptosis is not a reliable indicator of cellular sensitivity to anticancer agents. The message from Figure 3 is that cells can die from apoptosis at different rates, and that the dominant mode of cell death may, or may not be, apoptosis.

The same conclusion is also likely to hold for tumors in vivo. As noted above, the HCT116 $p21^{-/-}$ colon carcinoma cells are sensitive to radiation when growing as tumors in vivo. This is associated with a massive extent of apoptosis after irradiation (>90%) of the tumor cells as determined by the terminal deoxynucleotidyltransferase-mediated nick end labeling assay. This apoptosis can be almost completely eliminated by overexpressing Bcl-2 in these cells. Surprisingly, however, this does not affect the response of the tumors to radiation in vivo.

Of note from the data in Figures 1 and 3 is that the sensitivity of cells to killing as assessed by their ability to form a colony is greater than that for the short-term assay that measures the proportion of cells undergoing apoptosis. In other words, for a given toxic insult, more cells are killed when assessed by clonogenic assay than undergo apoptosis. This has been noted previously by several investigators (19, 37, 38), but it is contrary to the widely held view that the traditional mode of cell killing by radiation and anticancer drugs requires a greater amount of DNA damage than is required for apoptotic death. However, it is important to be aware of the fact that it can be difficult in some circumstances, particularly for tissues and tumors in vivo, to assess apoptosis, because it is a dynamic process and not all of the cells destined to die by apoptosis may be visible at any one time (37, 39).

These data lead to the conclusion that the response of tumor cells either in vitro or in vivo to genotoxic damage may, or may not, correlate with the extent of apoptosis after exposure. In the next section, we examine one particular aspect of genotype and sensitivity, i.e., the impact of wild-type or mutant p53 on cellular sensitivity to genotoxic damage.

**Does p53 Status Affect the Sensitivity of Cells of Nonhematological Origin to Genotoxic Damage?** Although there are many studies of the influence of p53 status on the sensitivity of cells to genotoxic agents, in view of the above discussion concluding that short-term assays can be misleading, we have considered only those studies that have used clonogenic survival (which is not affected by the rate of cell death). We have also focused primarily on those investigations using ionizing radiation. This is because different anticancer drugs have different mechanisms of action that could involve p53 directly independent of apoptosis (e.g., involving nucleotide excision repair), and because investigators use a wide variety of exposure conditions for drugs but use much more defined radiation exposure conditions. There are also more studies with ionizing radiation than with all anticancer drugs combined.

In reviewing the literature on the question of the influence of p53 on the radiation response of cells, we have therefore applied the following criteria:

(a) Only cells or tissues of nonhematological origin have been considered.

(b) Only investigations in which clonogenic survival was used to assess cell killing were considered.

We have also excluded from the analysis studies in which wild-type p53 was massively overexpressed in cells using viral vectors before irradiation. Typically, this results in radiation-induced apoptosis and radiation sensitization (40, 41).

The data in the literature conforming to these criteria fall into two main categories: (a) First are those papers in which a group of nongenetically matched cell lines have been assessed for statistical differences in radiation sensitivity between those that have wild-type p53 and those that have mutant p53. Of the 27 publications in the total pool, 10 fall into this category, of which 3 find p53 mutated cells more radiation resistant (42–44), 3 find p53 mutations make no difference to radiation sensitivity (45–47), and 4 find p53 mutated cells more radiation sensitive (48–51). Illustrative data from the two largest published series, each assessing radiation sensitivity and p53 status...
in cell lines or primary cultures from human cancers, are shown in Fig. 4. The second category includes publications in which investigators have used genetically matched cell lines in vitro or tissues in vivo from p53+/+ versus p53−/− mice, or have used cells with or without expression of the HPV protein E6, or have used transfection of mutant p53 or conditionally expressed p53. In this group, five investigations found p53 null/mutated cells more radioresistant (52–56), nine found no difference in radiation sensitivity between p53 wild-type or null/mutant cells (55, 57–64), and three found p53 null or mutant cells more sensitive than wild-type cells (59, 65, 66). It should be noted here that three of the five studies showing that loss of p53 function results in radioresistance used overexpression of mutant p53 in a p53 wild-type background (52–54). In a recent reanalysis of the largest of these studies, the resistance of the mutant p53-expressing cells was correlated with more proficient rejoining of DNA double-strand breaks (67). However, there was considerable overlap in sensitivity between the wild-type and mutant-expressing cells, and significant resistance did not occur until mutant p53 was overexpressed more than 100-fold.

Clearly, these data do not justify the conclusion that p53 mutated or null cells are in general more resistant to radiation-induced cell kill than are p53 wild-type cells.

For anticancer drugs, there have been surprisingly few studies using clonogenic survival to determine the influence of p53 on the sensitivity of cells of nonhematological origin. However, when this assay has been used, loss of wild-type p53 has been found to have no effect on the sensitivity of cells to the topoisomerase I inhibitor, camptothecin (61), doxorubicin (19, 68), or to taxol or vincristine (68). Interestingly, mutations in p53 have been shown to confer sensitivity to drugs whose toxicity is modulated by nucleotide excision repair, such as nitrogen mustard and cisplatin (38, 68). The study with murine teratocarcinoma cells is of particular interest because the absence of p53 protected against cisplatin-induced apoptosis, yet sensitized to cell kill determined by colony formation (38). This can be readily understood in terms of the drug doses used: to reach 90% cell kill by apoptosis in the most apoptotically sensitive cell line required some 50 times more cisplatin exposure than to produce an equal level of cell kill by clonogenic assay in the same cell line.

Given the above evidence that p53 status does not generally affect the sensitivity of cells to radiation and anticancer drugs, it is pertinent to ask how such a belief arose. Several factors have probably contributed.

(a) Lymphocytes, thymocytes, and lymphoma cells, are highly sensitive to anticancer therapy and invariably die a rapid apoptotic death that is dependent on wild-type p53 (3, 69).

(b) Short-term assays, rather than clonogenic survival, have often been used to assess cell killing. These can be markedly affected by the rate at which cells die, which, in turn, can be dependent on the mode of cell death (e.g., apoptosis or necrosis), and on the cellular genotype.

(c) As mentioned earlier, conclusions derived from normal cells transformed with dominant oncogenes have been extrapolated to tumor cells. We point out in the following section that these transformed normal cells are hypersensitive both to apoptosis and to killing as measured by clonogenic survival. The hypersensitive phenotype likely results from a synergy between oncogene activation of p53 and the DNA damage response and is not the case for tumor cells in general.

(d) The highly significant correlation of mutated p53 with resistance to active anticancer agents (that act principally by damaging DNA) in the National Cancer Institute screen of 60 different cell lines (17) is actually a correlation, not of cell kill, but of 2-day growth inhibition, with p53 status. Because wild-type p53 is required for growth arrest after DNA damage (70), such a correlation of sensitivity to growth inhibition with wild-type p53 is expected. Because there is little or no correlation of growth inhibition with cytotoxicity across
Fig. 4. p53 status does not affect the sensitivity of human tumor cells to killing by ionizing radiation. Cell sensitivity (surviving fraction after 2 Gy) was assessed by colony formation. Results shown in A are from primary tumor cell lines derived from head and neck cancers (46). In these studies, the p53 gene was sequenced to determine those tumors expressing wild-type or mutant p53. Results in B show sensitivity of p53-expressing versus p53-nonexpressing primary cultures from ovarian cancers (●) and melanoma (▲; 45). In these studies, high levels of p53 expression were used as a surrogate for p53 mutation. ♦, mean; bars, ± 1 SE for each population.

There are other situations in which apoptosis can contribute to overall sensitivity have in large part been those that used cells of nonlymphoid origin, the cases in which apoptosis does contribute to overall sensitivity of lymphoid cells: death is usually rapid after treatment, is dependent on the status of p53 (16, 79), and can be inhibited by overexpression of bcl-2 (72, 80, 82). For example, in MEFs transformed with E1A and Ha-ras, cells with wild-type p53 undergo rapid apoptosis in response to genotoxic and nongenotoxic stress, whereas similarly transformed cells from p53 knockout mice do not (16, 82). This dramatic difference in apoptosis also translates into an increased sensitivity of the transformed p53<sup>+/+</sup> MEFs in terms of the overall sensitivity as determined either by clonogenic survival<sup>6</sup> or by tumor response in vivo (15).

The recent discovery of ARF, a protein encoded by an alternative reading frame within the INK4a locus (83) and subsequent studies into ARFs function, provide a possible explanation for the hypersensitivity to overall killing observed in these cell lines. ARF up-regulates p53 in response to oncogene activation, including E1A, which abrogates p53 function, providing a possible explanation for the hypersensitivity to overall killing observed in these cell lines. ARF up-regulates p53 in response to oncogene activation, including E1A (84, 85), and can be inhibited by overexpression of E1A (84). In the previously described p53<sup>-/-</sup> and p53<sup>+/+</sup> MEFs, E1A does not increase the resistance of synchronized cells to genotoxic and nongenotoxic stress (84). In these hypersensitive cells, modulation of overall cell killing occurs when one of the pathways is disrupted, e.g., loss of p53. It is important to realize that the loss of p53 in the transformed MEFs results not so much in resistance to treatment as much as it does in an elimination of the unusually hypersensitive response (in this case, due to apoptosis) that is found after expression of E1A. The p53 null transformed MEFS revert back to an overall sensitivity that is closer to the untransformed parental p53<sup>-/-</sup> wild-type cells. In other words, in normal MEFs, p53 modulates the hypersensitive phenotype induced by oncogenic activation. Interestingly, this synergy also appears to be lost in E1A transformed cells that have lost ARF but retain wild-type p53 (84).

An illustration of the role that apoptosis can play in minimally transformed cells was demonstrated recently using Rat1 cells engineered to conditionally express myc (72). In this study, induced

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6 Unpublished data.
expression of myc increased the in vitro radiation sensitivity to both apoptosis and clonogenic survival, and this increased sensitivity was inhibited by expression of bcl-2. When grown as tumors in mice, expression of myc enhanced the sensitivity of these cells to fractionated doses of radiation, and this was abrogated by expression of bcl-2, indicating that apoptosis was directly influencing tumor response to radiation. This experiment thus provides direct evidence that expression of oncogenes in cells originally isolated from normal tissues can induce an apoptotically sensitive phenotype that is hypersensitive to treatment with anticancer therapies. Because myc has also been shown to activate ARF (85), it is possible that the enhanced sensitivity observed in these cells and tumors is also due to a synergy between oncogene-dependent and DNA damage-dependent pathways to p53 induction and p53-dependent apoptosis.

It has been studies such as these using engineered minimally transformed cells that have, in large part, shaped the current opinion that apoptosis is also an important determinant of the response of human tumor cells to cancer therapy. As discussed earlier, it has led to the notion that a tumor cell with mutant p53 will be more resistant than the same cell with wild-type p53, or that increased expression of bcl-2 in cancer cells will be protective. Acceptance of this hypothesis assumes that cells derived from human tumors will behave similarly to the virally or oncogenically transformed normal cells described above. However, a clear distinction between tumor cells and genetically engineered cells from normal tissues is the requirement of the tumor cells, during their evolution, to overcome any apoptotically sensitive state that may have been induced by initial oncogenic transformation. The selection against this apoptotically sensitive state is driven by the selective forces produced by microenvironmental stresses such as hypoxia (82), reduced growth factor and nutrient supply (76), and the requirement for anchorage independent survival (88, 89; Fig. 5).

It is, therefore, likely that the majority of tumors have evolved past the point where they may have been apoptotically hypersensitive to genotoxic and nongenotoxic stress. In explanted MEFs expressing viral or cellular derived oncogenes such as E1A or myc, this selection occurs rapidly, and elimination of the sensitive apoptotic state occurs by loss of either p53 or ARF. In human tumors, this phenotypic evolution can occur with or without alteration of genes such as p53 or those in the bcl-2 family but is associated with a loss of the rapid induction of apoptosis after genotoxic damage. This selection does not necessarily eliminate the ability of the cell to carry out apoptosis altogether; in some cases, the majority of tumor cells may still die by this process, although usually in a more delayed manner occurring several days after treatment and usually after cell division (23, 26, 90). Selection against the apoptotically sensitive phenotype is likely the explanation for our findings in the present review that apoptosis in human tumor cells has minimal impact on the overall cellular sensi-
Activity to present anticancer therapy. Several of these studies showed that even in cases where the level of apoptosis can be modified in human tumor cell lines by \(p53\) (60, 62), \(p21\) (22, 23), or \(bcl-2\) (20, 21, 36), the overall sensitivity to various genotoxic agents was unrelated to the level of apoptosis. This finding indicates that in these cases, apoptosis after genotoxic treatment occurs only in cells that have already lost their capacity to continue growth, likely as a result of unrepaired or misrepaired DNA lesions (Fig. 6).

The studies with oncogenically transformed normal cells, however, do suggest the exciting possibility of improving cancer therapy by exploiting apoptosis. Development of therapeutic agents that are able to revert human tumors to an apoptotically sensitive phenotype associated with initial transformation, e.g., by reactivation of pathways like the one described above in which ARF participates, would allow use of current cancer therapies with improved therapeutic potential. The key to the development of such therapies is an increased understanding of the genetic changes that occur both during initial oncogenic transformation as well as during the selection process that follows. However, equally important will be the use of assays that correctly identify therapies that affect the overall level of cell kill as opposed to those that simply measure changes in the kinetics or mode of cell death.

Summary

In this review, we have focused on the specific question of whether the sensitivity of cancer cells from nonhematological malignancies to anticancer drugs and radiation is affected by their \(p53\) status and/or their ability to undergo apoptosis after exposure. We find that if clonogenic survival is used as the end point for cell killing, neither \(p53\) status nor the ability of the cells to undergo apoptosis appears to play a significant role in the sensitivity of these cells to DNA-damaging agents. This conclusion is contrary to the widely held tenet that tumor cells with mutations in \(p53\) and/or that are resistant to apoptosis are also resistant to cancer treatment. Two principal factors have led to this view: (a) Many studies have used short-term assays, rather than clonogenic survival, to assess cell killing. Because apoptosis, particularly \(p53\)-dependent apoptosis, can occur rapidly after exposure, short-term assays tend to underestimate overall killing for cells with mutant \(p53\) or that do not undergo apoptosis. (b) To use
genetically matched cell lines, investigators have often used normal cells (differing in one specific gene such as p53) transformed with dominant oncoprogens as models of cancer cells. Such cells are unusually apoptotically sensitive compared with cancer cells, which have invariably undergone a selection process resulting in loss of this hypersensitive phenotype. Extrapolation of data from experiments involving such minimally transformed normal cells to cells from solid human tumors has thus exaggerated the importance of apoptosis as a determinant of treatment sensitivity.

Our conclusions that apoptosis and the genes affecting apoptosis such as p53 and members of the Bcl-2 family may not contribute significantly to the sensitivity of cancer cells to anticancer agents does not mean that these genes do not affect the prognosis of human tumors. Tumors with mutated p53 for example can be more anaplastic, have a higher rate of proliferation, and have a more aggressive phenotype than similar tumors with wild-type p53, thereby giving rise to a worse prognosis. Our conclusions also do not apply to cancers of hematological origin for which apoptosis appears to be the dominant form of cell death after exposure to anticancer agents, nor do they apply to death receptor (e.g., Fas)-mediated apoptosis. Finally, the fact that apoptosis is a critical determinant of treatment sensitivity in minimally transformed normal cells warrants further research efforts to understand the loss of apoptotic sensitivity that invariably occurs during solid tumor evolution in hopes of providing more effective cancer therapy.

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